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Mapping of Linear Antigenic Determinants on Glycoprotein C of Herpes Simplex Virus Type 1 and Type 2 Recognized by Human Serum Immunoglobulin G Antibodies

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Using membrane-based dekapeptides, the reactivity of human serum antibodies with linear antigenic determinants of herpes simplex virus (HSV) type 1 and type 2 glycoprotein C (gC-1, gC-2) was studied by pep scan and immunodot assay. The entire coding sequences of gC-1 and gC-2 were screened for the presence of linear epitopes by pep scan. Peptides recognized in an HSV-1 type-specific manner were mainly identified within the N-terminal third and at the C-terminus of gC-1, whereas most type-common antibodies were directed against colinear peptides within the central parts of gC-1 and gC-2. The type-specific reaction of human sera with gC-2 peptides in pep scan was poor. Eight peptides identified as immunoreactive by pep scan were further tested in immunodot assay for their reactivity with a human serum panel. None of the eight HSV-negative sera gave positive results by immunodot assay. Positive reactions with gC peptides were found to be strongly age-dependent, i.e., the rate of positive reactions was significantly higher in HSV-positive adults than in HSV-positive children. Antibody reactivity with two type-common gC peptides was demonstrated in 17 out of 28 HSV-positive sera. A putative type-specific gC-2 peptide employed in immunodot assay was inconsistently recognized by human sera. Twenty HSV-positive sera reacted with at least 1 of 5 type-specific gC-1 peptides. Nine sera showing no reactivity with glycoprotein G of HSV-1 (gG-1) by immunoblotting recognized type-specific gC-1 peptides in immunodot assay. Thus, gC-1 peptides might allow the detection of HSV-1-specific antibodies in individuals showing no reactivity with commonly employed HSV-1-specific diagnostic antigens, i.e., purified or recombinant gG-1. ***J. Med. Virol. 55:281–287, 1998.*** © 1998 Wiley-Liss, Inc.

KEY WORDS: HSV-1; HSV-2; glycoprotein C; human antibodies; linear antigenic determinants

INTRODUCTION

Although herpes simplex viruses type 1 and type 2 (HSV-1, HSV-2) do not strictly require glycoprotein C (gC) for viral multiplication in cell culture [Zezulak and Spear, 1984], gC plays an essential role in the pathogenesis of HSV infections in the human host. Thus, glycoprotein C is used by the virus to evade the complement-mediated attack [Friedman et al., 1996] and it is important for viral attachment [Kühn et al., 1990; Sears et al., 1991; Tal-Singer et al., 1995]. In addition, glycoproteins C of HSV-1 and HSV-2 (gC-1, gC-2) act as major antigenic determinants of the antiviral immune response [Ashley et al., 1985; Glorioso et al., 1985; Kühn et al., 1987].

The overall structure of gC and its disulfide bond arrangement seem to be similar in HSV-1 and HSV-2 [Rux et al., 1996]. Despite an amino acid identity of approximately 69% [Dowbenko and Lasky, 1984], gC-1 and gC-2 differ in their ability to bind C3b on infected cell surfaces, in their effect on the decay of the alternative pathway C3 convertase, and in their function in viral entry [Hung et al., 1994; Gerber et al., 1995]. Using monoclonal antibody-resistant (MAR) mutants, two major antigenic sites, termed antigenic site I and II, have been identified. These antigenic sites are in similar location on gC-1 and gC-2, respectively, and are

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structurally independent of each other [Wu et al., 1990; Dolter et al., 1992].

Both glycoproteins have been reported to be recognized type-specifically mainly by monoclonal and polyclonal antibodies. Cross-reactive antibodies exist, however, particularly in polyclonal antisera [Zweig et al., 1983; Koga et al., 1986; Dolter et al., 1992], thus limiting the use of gC as a type-specific antigen for serodiagnosis. In contrast to gC, glycoproteins G of HSV-1 and HSV-2, gG-1 and gG-2, do not elicit a cross-reactive humoral immune response during natural infection [Ashley et al., 1988]. Therefore, gG-1 and gG-2 are widely employed as type-specific antigens in immunoassays [Bergström and Trybala, 1996]. Nevertheless, a number of arguments exist that make gC attractive as diagnostic antigen. Thus, gC is immunodominantly recognized by human sera, and antibodies against gC and glycoprotein B (gB) were found to be the first to appear during primary infection [Ashley et al., 1985]. In addition, gC-1 and gC-2 share only limited sequence homology within their N-terminal part. The N-terminus of gC-1 contains a stretch of 28 amino acid residues that is absent from gC-2 [Frink et al., 1983; Swain et al., 1985].

In order to localize type-common and type-specific epitopes on gC recognized by human sera, a membrane-based pep scan technique was used to screen the entire coding sequences of gC-1 and gC-2 for the presence of potential linear antigenic sites. Sites identified by pep scan were further tested for their reactivity with a panel of human sera by immunodot assay. Our results indicate that the use of gC peptides might be an interesting alternative to type-common and type-specific HSV antigens commonly used in immunoassays.

MATERIALS AND METHODS

Pep Scan and Immunodot Assay

Using the SPOTs system (IC-Chemicals, Ismaning, Germany), overlapping dekapeptides (9 amino acids overlapping) representing the entire coding region of gC-1 (strain KOS, 511 amino acids, 502 dekapeptides) [Frink et al., 1983] and gC-2 (strain 333, 480 amino acids, 471 dekapeptides) [Swain et al., 1985] were synthesized on derivatized cellulose membranes in an 8 × 12 matrix of small circular dots according to the manufacturer's instructions. For analysis by immunodot assay, peptides identified as reactive by pep scan were resynthesized on strips of cellulose membranes. The homology between gC-1 and gC-2 peptides was determined using the sequence analysis software Mac Vector 6.0 (Integra Biosciences, Fernwald, Germany).

Recognition of peptides by human serum antibodies was detected as follows. After washing with Tris-buffered saline (TBS) pH 8.0 for 10 min at room temperature (RT), and overnight incubation at RT in blocking buffer (concentrated membrane blocking buffer (IC-Chemicals) diluted 1:10 in T-TBS (TBS pH 8.0 containing 0.05% Tween 20) with 5% sucrose (w/v)), membranes were washed for 10 min in T-TBS and incubated with human sera diluted 1:100 in blocking buffer for 4 hr at RT. Membranes were then washed three times with T-TBS. Specifically bound antibodies were detected by incubation of membranes for 2 hr with rabbit antihuman antibodies (Medac, Hamburg, Germany) diluted 1:400 in blocking buffer, followed by three washes and incubation for 2 hr with β-galactosidase-conjugated goat antirabbit antibodies (IC-Chemikalien, Ismaning) diluted 1:200 in blocking buffer. Subsequently, membranes were washed two times with T-TBS, and twice with phosphate-buffered saline (PBS) pH 7.0. After addition of substrate solution (10 ml PBS containing 4.9 mg BCIG (5-bromo-4-chloro-3-indolyl-β-D-galactopyranoside dissolved in 100 μl dimethyl formamide), 1 mM $MgCl_2$, 10 mM potassium ferricyanide), the color reaction was allowed to develop until a clear distinction between positive and negative peptide dots could be made. The reactivity of peptides with human antibodies was measured by densitometric scanning and computer-assisted processing of data. Briefly, the signal intensity within the center of individual peptide dots was measured (in relative units), background activity as determined at the margin of each dot was subtracted, and the reactivity index, i.e., the quotient of signal intensity obtained with HSV-positive sera divided by the signal intensity of the second antibody control, was calculated. In pep scan, peptides were classified to be specifically recognized by human serum antibodies if their reactivity index was >5. Few peptides reacted strongly with the second antibody controls in pep scan (data not shown). These peptides were considered to be recognized nonspecifically and excluded from further analyses by immunodot assay. In immunodot assay, the signal intensity of individual dots was determined by densitometrical scanning as described above. Dots were considered to be recognized specifically if their signal intensity exceeded the mean plus threefold standard deviation of eight HSV-negative control sera.

Immediately after documentation of the color reaction, membranes were regenerated according to the manufacturer's instruction and blocked overnight or stored at −20°C until use. In general, 8 to 10 immunostaining reactions could be carried out with each membrane before fading of the peptide reactivity occurred.

Human Sera

A total of 42 human sera were tested for reactivity with gC-peptides. Twenty-eight sera stemmed from patients attending the University Hospital of Cologne for reasons other than acute HSV infection (15 pediatric patients, mean age 11 years, and 13 sera from adult patients, mean age 30 years), the other 14 sera came from female prostitutes (mean age 22 years). Prior to analysis in pep scan or immunodot assay, respectively, human sera were tested for the presence of HSV-specific IgG antibodies by ELISA (HSV-Enzygnost, Behringwerke, Marburg, Germany), by microneutralization assay, and by immunoblotting using purified

HSV-1 and HSV-2 virions as described previously [Kühn et al., 1987].

Antibody reactivity with gG-1 and gG-2 in immunoblot was used to demonstrate the presence of HSV type-specific antibodies in human sera. Bands corresponding to gG-1 and gG-2, respectively, were identified in immunoblots using a gG-1-specific rabbit serum and a gG-2-specific monoclonal antibody (the latter was a kind gift of Tomas Bergström, Göteborg). Additionally, the reactivity of sera with gC-1 in immunoblotting was documented. The position of gC-1 in immunoblots was identified using monoclonal antibody IV4.1 [Kühn et al., 1990].

RESULTS AND DISCUSSION

Classification of Human Sera

Eight out of 42 sera, all of which stemmed from pediatric patients, were found to be HSV antibody-negative. Of the remaining 34 HSV-positive sera, 15 sera reacted in an HSV-1 type-specific manner in immunoblot (HSV-1 sera), 3 sera reacted HSV-2 type-specifically (HSV-2 sera), and 8 sera were found to contain HSV-1- and HSV-2-type-specific antibodies (HSV-1/HSV-2 sera). In 8 HSV-positive serum samples, analysis by immunoblotting failed to demonstrate type-specific antibodies (HSV-positive, nontypable). Seven out of 14 sera from female prostitutes and 3 out of 13 sera from adult patients without symptoms of acute HSV-infection contained HSV-2 type-specific antibodies, whereas none of the HSV-positive sera from pediatric patients reacted with gG-2. Antibodies directed against gC-1 were detected by immunoblotting in all HSV-1 sera, HSV-2 sera, HSV-1/HSV-2 sera, and 6 out of 8 HSV-positive, nontypable sera.

Detection of Linear Antigenic Determinants on gC by Pep Scan

In the first part of the study, gC-1 and gC-2 were screened for the presence of potential linear antigenic sites recognized by polyclonal human antibodies. For this purpose, 6 HSV-positive sera, in particular, 3 HSV-1 sera (2 adult patients and 1 female prostitute) and 3 HSV-1/HSV-2 sera (1 adult patient and 2 female prostitutes) were tested by pep scan for reactivity with membrane-based, overlapping dekapeptides representing the entire protein sequences of gC-1 and gC-2, respectively.

To allow a better comparison of the antibody reactivity with gC-1 and gC-2 in pep scan, the peptide sequences were aligned, and the number of positive reactions with each peptide, as well as the mean intensity of signals, was separately determined for HSV-1- and HSV-1/HSV-2 sera (summarized in Fig. 1).

Peptides were classified as type-common if HSV-1 sera reacted with the corresponding HSV-1 and HSV-2 dekapeptides. Peptides located within the N-terminal stretch of gC-1 showing no homology to gC-2 (Fig. 1) were considered to be HSV-1 type-specific. In addition, gC-1 peptides were classified as HSV-1 type-specific if HSV-1 and HSV-1/2 sera failed to react with the corresponding gC-2 peptides. Accordingly, gC-2 peptides were classified as type-specific if they were recognized exclusively by HSV-1/HSV-2 sera.

All sera exhibited a complex and individual pattern of reactivity with multiple gC-1 and gC-2 peptides in pep scan. Nevertheless, the overall pattern of antibody reactivity of HSV-1 and HSV-1/HSV-2 sera with gC-1 and gC-2 peptides appeared to be similar. Thus, prominent signals were obtained with peptides that were colinearly located within the central part of the external domain of both proteins, representing gC-1 amino acid residues 241–257, 295–310, and 396–408, and gC-2 amino acid residues 210–226, 264–279, and 365–377, respectively (Fig. 1). Since the HSV-1 sera reacted with the corresponding gC-1 and gC-2 peptides, these potential antigenic sites were considered to be recognized in a type-common manner.

Within the N-terminal part of gC, prominent signals were obtained with peptides corresponding to the gC-1 amino acid residues 15–30, 97–113, and 123–42. According to the criteria given above, these potential antigenic sites were considered to be recognized in an HSV-1-specific manner.

In contrast to gC-1, only few peptides from the N-terminal portion of gC-2 were found to be consistently recognized by human antibodies. Peptides corresponding to the gC-2 amino acid residues 42–55 strongly reacted with a single serum out of three sera classified as HSV-1/HSV-2-positive, and were thus tentatively identified as potential candidates for an HSV-2 type-specific antigenic site. Another potential antigenic site within the N-terminal part of gC, i.e., peptides corresponding to gC-2 amino acid residues 126–137 and the homologous gC-1 amino acid residues 158–167, was recognized also by HSV-1 sera, therefore most likely representing a cross-reactive, type-common epitope (Fig. 1).

Within the C-terminal part of gC, gC-1 peptides corresponding to amino acid residues 428–441 were considered to be recognized in a type-specific manner by human antibodies. Furthermore, 2 out of 3 HSV-1 sera recognized peptides within the divergent, highly charged intracytoplasmic tail of gC-1, and 1 out of 3 HSV-1/HSV-1 sera reacted with the corresponding gC-2 peptides (Fig. 1).

According to the method of Hopp and Woods [1981], gC-1 amino acid residues 127–132, 465–470, 506–511 and gC-2 amino acid residues 217–222, 272–277, and 434–439 were predicted as antigenic determinants. By pep scan, human sera reacted strongly with the majority of these predicted epitopes (Fig. 1). Thus, amino acids 127–132 of gC-1 were found to be part of a longer stretch of peptides that was recognized by all sera tested by pep scan. Amino acid residues 506–511 were found to be contained within a type-1-specific peptide (2 out of 3 HSV-1 sera positive) (Fig. 1). Amino acids 217–222 and 272–277 of gC-2 were part of peptides

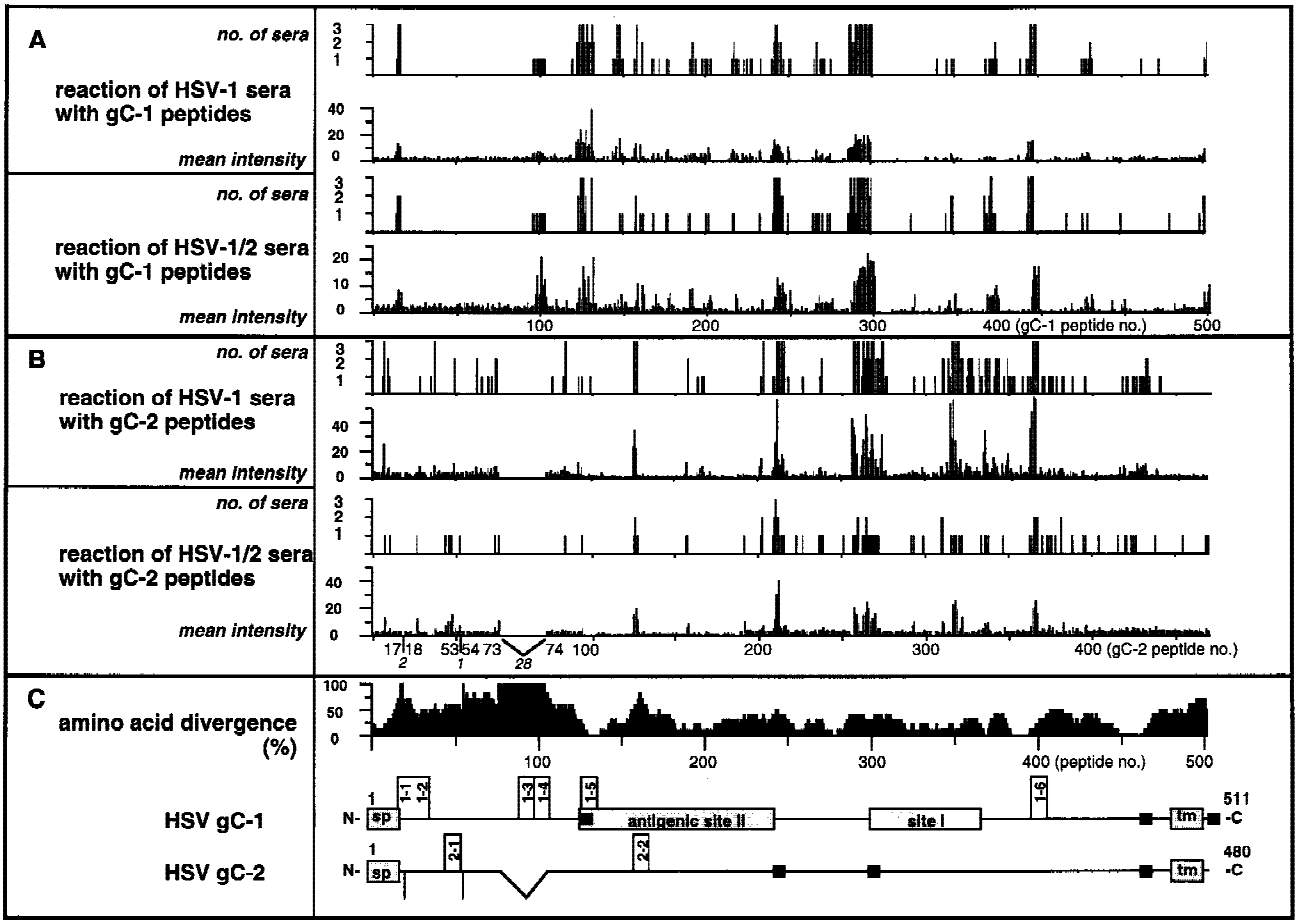

Fig. 1. Reactivity of human polyclonal sera with overlapping dekapeptides representing the entire coding sequences of HSV gC-1 and gC-2. The number of positive reactions of three HSV-1 and three HSV-1/2 sera, respectively, and the mean intensity of antibody reaction (sum of reactivity indices divided by the number of sera tested, calculated as given in the text) with each gC-1 dekapeptide **(panel A)** and gC-2 dekapeptide **(panel B)** are shown. For better comparison of results, the amino acid sequences of gC-1 and gC-2 were aligned; numbers refer to gC-1 and gC-2 dekapeptides, respectively. Three gaps were introduced within the gC-2 sequence to allow alignment of gC-1 (511aa) and gC-2 (480aa), and the number of intervening amino acid residues solely occurring in gC-1 (2, 1, and 28 aa, respectively, given in italics) are shown in panel B. The structure of gC-1 and gC-2 is schematically depicted in **panel C.** The degree of amino acid divergence among homologous gC-1 and gC-2 peptides is given in percentage (numbers refer to the position of gC-1 peptides), gaps introduced within the gC-2 sequence for alignment are indicated. The location of predicted antigenic determinants on gC-1 and gC-2 is shown by filled squares; sp denotes signal peptide; tm, transmembrane domain; antigenic sites I and II, major antigenic sites defined on gC by mononclonal antibodies [Wu et al., 1990; Dolter et al., 1992]. The position of peptides C1-1 to C1-6, C2-1, and C2-2 used in immunodot assay (see also Tables I and II) is indicated by numbered boxes (1–1 to 1–6, 2–1, and 2–2).

reactive in a type-common manner (Fig. 1). The prominent antibody response in pep scan against peptides partially overlapping with the signal peptide sequence of gC-1 was an unexpected finding since the antigenicity of this region of gC was predicted to be low.

As expected by the different experimental approach used, the distribution of linear antigenic determinants recognized by human antibodies only partially corresponded to the major antigenic sites I and II of gC-1 and gC-2, which had been previously defined by mononclonal antibodies [Wu et al., 1990; Dolter et al., 1992]. In pep scan, antigenic determinants clustered mainly within the regions flanking antigenic sites I and II of gC-1 and homologous parts of gC-2, respectively (Fig. 1). Many of these antigenic determinants recognized in a type-common manner by human sera consisted of relatively long stretches of amino acid residues. Therefore, they most likely represent a combination of several overlapping linear epitopes rather than single antigenic determinants.

The results show, however, that antibody responses in humans may also be elicited by the N-terminus and C-terminal intracytoplasmic domain of gC. The relatively low reactivity of human antibodies with the highly divergent portion of the N-terminus of gC-1 might reflect the clustering of O-linked carbohydrates in this region [Olofsson et al., 1991; Rux et al., 1996].

Analysis of Human Sera by Immunodot Assay

According to the results of the pep scan, six gC-1 dekapeptides (designated C1-1 to C1-6; Table I and Fig. 1) comprising amino acid residues 16–25, 21–30, 87–96, 99–108, 126–135, and 398–407, respectively, and

TABLE I. HSV-1 and HSV-2 gC Peptides Employed in Immunodot Assay

Designation	Position on gC	Amino acid sequence[a]
C1-1	gC-1 16–25	**L W L G** A G **V** A G G
C1-2	gC-1 21–30	G **V** A G G S E T **A S**
C1-3	gC-1 87–96	**T** P K **P** T **S T** P K S
C1-4	gC-1 99–108	T S T P D P K P K N
C1-5	gC-1 126–135	W **C** D **R R D P L A R**
C1-6	gC-1 398–407	**A W F L G D D** P **S P**
C2-1	gC-2 43–52	N **A** A P **S** A **S P** R N
C2-2	gC-2 127–136	**I W R Y A** T A T D A

[a]Homology between corresponding gC-1 and gC-2 peptides is indicated as follows. Identical amino acid residues are given in bold and are underlined, similarities are indicated in bold, and mismatches are shown in normal letters.

two gC-2 peptides (designated C2-1 and C2-2; Table I and Fig. 1) comprising amino acid residues 43–52 and 127–136, respectively, were further tested for reactivity with human sera by immunodot assay. Thirty-six human sera, including three sera previously studied by pep scan, were available for testing by immunodot assay.

Analysis of the reactivity of eight HSV-negative sera derived from pediatric patients demonstrated that the membrane-based peptides employed in the immunodot assay differed in their level of background activity. In order to minimize false negative and false positive results, an individual cutoff was calculated for each peptide (mean reactivity of HSV-negative sera plus threefold standard deviation). By this approach, none of the HSV-negative sera showed signals above the cutoff by immunodot assay, whereas positive reactions of HSV-positive sera could be clearly demonstrated.

In addition to the peptides listed in Table I, gC-1 peptides corresponding to the amino acid residues 247–256 and 297–306 were tested for reactivity with human sera by immunodot assay. These peptides, which strongly reacted in a type-common manner with the majority of human sera in pep scan (Fig. 1), also gave positive signals, however, with some of the HSV-negative control sera from pediatric patients in immunodot assay (data not shown). They were therefore excluded from further analyses by immunodot assay.

Table II summarizes the reactivity of sera with gC-1 and gC-2 peptides by immunodot assay. Of all peptides tested, peptide C1-5 showed the highest number of positive reactions, e.g., 18 out of 28 HSV-positive sera. Furthermore, the mean signal intensity obtained in positive sera was found to be highest with peptide C1-5 (data not shown). According to the results of pep scan, peptides C1-1 to C1-5 were classified as HSV-1 type-specific. Twenty HSV-positive sera reacted with at least one of these peptides, including 9 sera showing no reactivity with gG-1 in immunoblot , i.e., 3 HSV-2-positive and 6 HSV-positive nontypable sera (Table II). This finding suggested that the putative type-specific gC-1 peptides C1-1 to C1-5 may be at least partially recognized in a type-common manner by human serum antibodies. Several lines of evidence exist, however, to suggest that recognition of these peptides by human antibodies most likely indicates the presence of HSV-1-specific antibodies. Thus, four of the putative type-1-specific peptides that were recognized by all HSV-2-positive and 5 out of the 6 HSV-positive nontypable sera were found to share either no (C1-4) or only limited (C1-1 to C1-3) sequence homology with gC-2 (Table I). It appears to be unlikely, therefore, that a cross-reactive immune response is directed against these peptides. Furthermore, seroepidemiological studies have demonstrated that "pure" HSV-2 infections rarely occur in adults and that the number of HSV-1 and HSV-1/HSV-2 double infections almost equals the total number of HSV-positive individuals (for review, see Nahmias et al. [1990]). Due to the relatively low sensitivity of gG-1 as diagnostic antigen [Bergström and Trybala, 1996], the classification of sera according to their reactivity with gG-1 by immunoblots might have led to an underestimation of the number of sera containing HSV-1-specific antibodies in our study. It appears likely, therefore, that the majority of sera classified as HSV-positive nontypable and "solely" HSV-2-positive contain HSV-1-specific antibodies.

In contrast to the peptides C1-1 to C1-4, the putative type-1-specific peptide C1-5 was found to have 70% amino acid identity to gC-2 (Table I). Dolter et al. [1992] demonstrated that type-specific mononoclonal antibodies against gC recognize areas possessing substantial homology between both molecules. Thus, a type-specific polyclonal humoral immune response might be also elicited by relatively well-conserved regions of gC.

Peptides C1-6 and C2-2, which had been classified in pep scan as type-common, were recognized by 17 out of 28 HSV-positive sera (Table II). The relatively low frequency of positive results with these peptides as compared to pep scan (all sera reactive, Fig. 1) is most likely due to the different cutoff approach used in immunodot assay. Although poorly recognized by human sera, peptide C2-1 was found to be mainly recognized in an HSV-2 type-specific manner. Thus, all HSV-2 sera, 1 out of 5 HSV-1/HSV-2 sera, and 1 out of 8 HSV-positive nontypable sera were found to react with this peptide. The latter serum exclusively recognized peptide C2-1 by immunodot assay and showed a broader reactivity with HSV-2 virion proteins as compared to HSV-1 proteins by immunoblotting.

As compared to the results of ELISA, microneutralization assay, and immunoblotting, the overall sensitivity of immunodot assay for the detection of HSV-specific antibodies was calculated to be approximately 80%, i.e., 22 out of 28 HSV-positive sera reacted with at least one of the peptides included in immunodot assay (Table II).

In general, sera stemming from adults showed a broader reactivity with gC peptides than sera obtained from children. Thus, 9 out of 11 HSV-positive sera from adult patients (ca. 82% sensitivity), and all sera from female prostitutes (100% sensitivity) recognized at

TABLE II. Reactivity of Human Sera With gC Peptides in Immunodot Assay

Sera	Reactivity with gC peptides[a]										
	C1-1 1-sp	C1-2 1-sp	C1-3 1-sp	C1-4 1-sp	C1-5 1-sp	any 1-sp	C1-6 tc	C2-2 tc	any tc	C2-1 2-sp	any gC peptide
HSV-negative sera (n = 8)											—
HSV-1 sera[b] (n = 12)	3	2			5	6	2	5	5		6
HSV-2 sera (n = 3)	3	2	1	2	3	3	3	3	3	3	3
HSV-1/HSV-2 sera (n = 5)	3	3	1	1	5	5	5	5	5	1	5
HSV-positive non-typable (n = 8)	2	3	1	2	5	6	3	3	4	1	7
gC-1-positive sera[b] (n = 26)	10	10	3	5	16	18	12	14	15	5	20
gC-1-positive, gG-1-negative sera (n = 9)	2	6	2	4	6	8	4	4	4	3	8
gC-1-negative, HSV-positive sera (n = 2)	1				2	2	1	2	2		2
HSV-positive pediatric patients (n = 7)				1	1	2	1		1		2
HSV-positive adult patients (n = 10)	2	4	2	2	6	7	7	6	6	2	9
HSV-positive female prostitutes (n = 11)	9	6	1	2	11	11	5	10	10	3	11
Total HSV-positive sera (n = 28)	11	10	3	5	18	20	13	16	17	5	22

[a]According to the results of pep scan, peptides were classified as HSV-1-specific (1-sp), type-common (tc), or HSV-2-specific (2-sp); any 1-sp and any tc denote sum of reactivity of sera with HSV-1-specific and type-common peptides, respectively.
[b]Sera were classified according to their reactivity with gG-1 and gG-2, respectively; the presence of antibodies directed against gG-1, gG-2, and gC-1 was demonstrated by immunoblotting as described in Materials and Methods.

least one of the peptides included in immunodot assay (on average ca. 4 peptides per positive serum reactive in immunodot assay). In contrast, only 2 out of 7 HSV-positive sera obtained from children (ca. 29% sensitivity) reacted with the gC peptides by immunodot assay (on average 1 to 2 peptides per reactive serum) (Table II).

The low or missing reactivity of sera from children with gC peptides may reflect the fact that the prevailing antibody response against gC is directed against conformationally dependent epitopes [Dolter et al., 1992]. Recurrent HSV-infections have been previously reported to lead to an increase in the number of HSV proteins recognized by immunoblotting [Kühn et al., 1987], most likely by increasing the titers of antibodies reactive with conformationally independent, strictly linear epitopes. Double infections with HSV-1 and HSV-2 may have similar effects on the antibody reactivity, thereby increasing the rate of positive reactions with gC peptides in adults.

Comparison of the gC-specific antibody response by immunoblot and immunodot assay demonstrated that the majority of HSV-positive sera recognizing gC-1 by immunoblot also reacted with type-specific gC-1 peptides in immundot assay, i.e., 18 out of 26 sera. Two sera showing no reactivity with gC-1, gG-1, or gG-2 by immunoblot also recognized the gC-1 peptides employed by immunodot assay (Table II). Six sera, all of which recognized gC-1 by immunoblot, remained negative by immunodot assay, i.e., 5 HSV-1 sera (4 pediatric patients, 1 adult) and 1 HSV-positive nontypable serum (pediatric patient). In these sera, antibodies directed against highly carbohydrate-dependent epitopes on gC-1 [Sjöblom et al., 1987] might be reponsible for positive reactions with gC-1 by immunoblotting.

In conclusion, the use of type-common and type-specific HSV peptides as antigens for the serodiagnosis of HSV-infections might be an interesting alternative to whole-cell lysates or purified virions commonly employed as antigens in ELISA, as well as to type-specific viral antigens, e.g., purified or recombinant glycoprotein G of HSV-1 and HSV-2, and type-specifically reactive segments of glycoprotein B of HSV [Ashley et al., 1988; Goade et al., 1996].

Analysis of the entire coding sequences of gC-1 and gC-2 by pep scan allowed the detection of type-common, linear antigenic determinants recognized by human sera. Peptides that were immunodominantly recognized in an HSV-2 type-specific manner by human sera, however, could not be identified on gC-2. This finding may reflect a low level of immunogenicity of type-specific, linear epitopes on gC-2. In contrast, several of the gC-1 peptides identified as immunoreactive by pep scan appear to be recognized in an HSV-1-specific manner and were demonstrated to react specifically with a high percentage of HSV-positive sera by immunodot assay in this study. They may in fact represent promising candidates for use in routine diagnosis. In combination with other type-specific peptides, they might allow the pinpointing of HSV-1-specific antibodies in dually infected individuals, which would have been otherwise overlooked on account of the relatively low sensitivity of gG-1 as type-specific antigen [Bergström and Trybala, 1996].

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